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The Ancient and Evolving Roles of Cohesin in Gene Expression and DNA Repair

Review

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The cohesin complex, named for its key role in sister chromatid cohesion, also plays critical roles in gene regulation and DNA repair. It performs all three functions in single cell eukaryotes such as yeasts, and in higher organisms such as man. Minor disruption of cohesin function has significant consequences for human development, even in the absence of measurable effects on chromatid cohesion or chromosome segregation. Here we survey the roles of cohesin in gene regulation and DNA repair, and how these functions vary from yeast to man.

Introduction

Cohesin is a member of a group of protein complexes that contain Structural Maintenance of Chromosome (SMC) proteins. Prokaryotes usually have one SMC complex that performs multiple roles in chromosome mechanics, while eukaryotes have multiple specialized complexes, including cohesin. The cohesin complex, which consists of a heterodimer of Smc1 and Smc3, the Rad21 (Mcd1/Scc1) kleisin protein, and Stromalin (SA, Scc3, Stag1/2) (Figure 1), has critical roles in sister chromatid cohesion, DNA repair, and gene regulation [1].

SMC proteins fold back on themselves in the 'hinge' region to form antiparallel coiled-coil arms, with the amino and caboxyl termini coming together in 'head' domains that contain *ABC*-type ATPases (Figure 1). Cohesin forms a ring-like structure, with Rad21 bridging the SMC head domains. The internal cohesin diameter is on the order of 35 by 50 nm, large enough to encircle two DNA molecules. Thus, the leading idea is that cohesin binds to chromosomes topologically, and that it mediates sister chromatid cohesion by one ring encircling both sisters, or by two rings, each encircling one sister, interacting with each other [1]. The experimental evidence that cohesin topologically entraps circular yeast minichromosomes is compelling, but the mechanism of cohesion remains unresolved [2,3].

Cohesin Binding and Chromosome Localization Differ between Organisms

Figure 1 outlines the cohesin chromosome binding cycle, reviewed in detail elsewhere [4]. Cohesin is loaded onto chromosomes in telophase in higher eukaryotes but at the G1/S boundary in *Saccharomyces cerevisiae*. Cohesin is loaded by a protein complex, recently dubbed kollerin [4], consisting of an adherin protein (Scc2, Mis4, Nipped-B, NIPBL) and the Scc4 (Mau-2) protein. Kollerin binds chromosomes and is required, along with ATP hydrolysis by the SMC proteins, for topological binding of cohesin [4–6]. The mechanism of cohesin loading is unknown, but evidence suggests that the ring may open at the Smc1/3 hinge dimer interface to permit DNA entry [4]. The Smc1/3 hinge dimer binds DNA, which may position it for loading [7].

Sister chromatid cohesion is established during S phase, in coordination with DNA replication, but the mechanisms are unknown [1,4,8]. Cohesion establishment requires specialized DNA replication factors and acetylation of Smc3 by Eco1 (Ctf7) in budding yeast. Two Eco1 orthologs in *Drosophila* (Deco, Sans) and mammals (Esco1, Esco2) also regulate cohesion. Smc3 acetylation counteracts an anti-cohesion establishment function of the releasin complex, formed by the Pds5 and Wapl (Wpl1, Rad61) proteins. Pds5 is also paradoxically required to establish and/or maintain cohesion. In vertebrates and *Drosophila*, acetylation of Smc3 recruits the Sororin (Dalmatian) protein, which protects cohesin from removal by displacing Wapl from the releasin complex [9]. Hos1 in yeast, and HDAC8 in human cells deacetylates Smc3 in preparation for the next cell cycle [4,10–12].

The process of cohesin removal for cell division differs between yeast and higher eukaryotes [1,4]. In yeast, Polo/cdc5 phosphorylates the Scc1 cohesin subunit, making it sensitive to proteolysis by separase upon its activation at the metaphase to anaphase transition. In higher eukaryotes cohesin removal is a two-step process, in which phosphorylation of the SA/Scc3 subunit (Stag1/2) by a Polo-like kinase stimulates cohesin removal from the chromosome arms in prophase, possibly driven by releasin. Shugoshin protein blocks removal of pericentric cohesin until activation of separase at the metaphase to anaphase transition [13].

Fluorescence recovery after photobleaching (FRAP) reveals that cohesin chromosome binding dynamics are complex during interphase, when gene regulation and DNA repair occur. There are multiple binding modes with chromosomal residence times ranging from seconds to hours [5,14,15]. Cohesin with a residence time of several seconds likely binds DNA directly without topologically entrapping it [7], while stable cohesin with a residence time of several minutes to hours is likely bound topologically. The residence time of stable cohesin is greater in G2 than in G1, indicating that it is further stabilized when mediating sister cohesion. In *Drosophila*, the amount of stable topological cohesin during interphase depends on the dosage of Nipped-B, Pds5 and Wapl, indicating that it is determined by a continuous balance between loading by kollerin and removal by releasin [5]. A fraction of both Nipped-B and Pds5 have the same unusually long residence time as topological cohesin, suggesting that kollerin and releasin can interact tightly with cohesin [5].

Cohesin binding is high around centromeres in all organisms, but there are intriguing differences in binding along chromosome arms. In *S. cerevisiae*, cohesin binding sites only partially overlap those for the Scc2 adherin, and most arm binding sites are located between convergently transcribed genes, giving rise to the ideas that cohesin slides from loading sites to the binding sites, and that it might be pushed there by RNA polymerase [16,17]. In the fission yeast *Schizosaccharomyces pombe*, cohesin co-localizes with the Mis4 adherin at highly expressed genes, and localizes between some, but not all, convergent genes in G2 [18,19].

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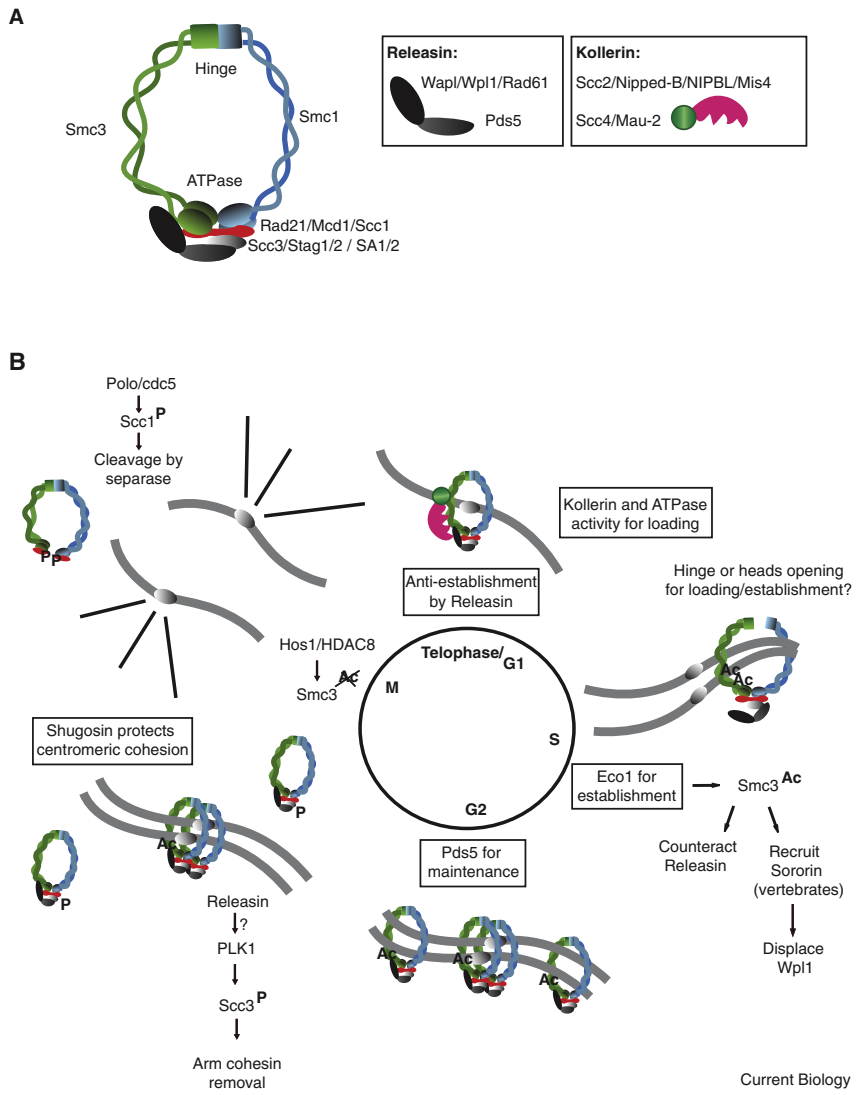

Figure 1. Cohesin structure and cell cycle regulation.

(A) A schematic representation of the cohesin complex and its subunits. (B) An overview of cohesin chromatin loading, and removal from chromosomes, as well as cohesion establishment during an unperturbed cell cycle. Main steps during the cohesion cycle and species differences are highlighted. For further details see the main text. Phosphorylation, P; Acetylation, Ac.

Drosophila shows a very different pattern. Cohesin and the Nipped-B adherin co-localize almost completely genome-wide, with the exception of meiotic centromeres, which bind cohesin but not Nipped-B [20,21]. In addition to DNA replication origins, Nipped-B and cohesin bind preferentially to a subset of active genes, with the highest levels at the transcription start sites, and are excluded from inactive or silenced genes [21–23].

Mammalian cells show a cohesin-binding pattern similar to that in *Drosophila*, except that cohesin, but not the NIPBL adherin, bind closely adjacent to a large fraction of the sites that bind the CTCF transcription factor [24–30]. CTCF interacts directly with the cohesin SA (Stag2) subunit, and thus may recruit cohesin directly, or trap cohesin that slides along the chromosome from loading sites [28,31]. This is potentially an important distinction, because if cohesin is recruited directly in the absence of adherin, it is unlikely to encircle DNA. *Drosophila* CTCF lacks the domain that interacts with cohesin, and does not co-localize with cohesin.

Different Cohesin Functions Require Different Amounts of Cohesin

In *S. cerevisiae*, as little as 13% of normal cohesin levels supports sister chromatid cohesion, but there are defects in DNA repair and chromosome condensation [32]. Reduction of cohesin levels by 80% in *Drosophila* cells has dramatic effects on gene transcript levels, but no significant effect on cohesion or chromosome segregation [33].

Even more strikingly, heterozygous loss-of-function mutations in the human *NIPBL* adherin gene, which reduce expression by 30% or less, cause Cornelia de Lange syndrome (CdLS), with severe effects on physical and mental development [34,35]. Individuals with CdLS grow slowly, and suffer cognitive deficits, autism and abnormalities in organs and limbs. Cells from CdLS patients show changes in gene expression and mild effects on DNA repair, without overt effects on sister cohesion [25,36,37]. Milder forms of CdLS are caused by dominant missense mutations in mitotic Smc1 (SMC1A), also with changes in gene expression, mild defects in DNA repair, but no cohesion defects [25,38–40]. Moderate reduction in the expression of *Drosophila Nipped-B*, *Smc1*, or *pds5*, zebrafish *rad21*, and mouse *Nipbl*, *Pds5A* or *Pds5B* results in significant effects on gene expression and development without effects on cohesion or chromosome segregation [41–47]. Gene expression changes occur upon depletion of cohesin in non-dividing cells, confirming that cohesin affects gene expression independently of its roles in cell division [48–50]. Cohesin can directly modulate transcription, given that the genes that change in expression upon cohesin depletion or mutation are highly enriched for cohesin-binding genes, and that effects on cohesin-binding genes can occur within a few hours of cohesin depletion [23–25,33].

Thus, gene expression and development are most sensitive to cohesin activity, followed by DNA repair, and then sister chromatid cohesion. It may be that cohesion is the most ancient and inflexible role of cohesin, and thus the most resistant to perturbation. As outlined below, current evidence reveals that cohesin regulates transcription by multiple organism- and context-dependent mechanisms, and plays multiple roles in DNA repair and genome stability.

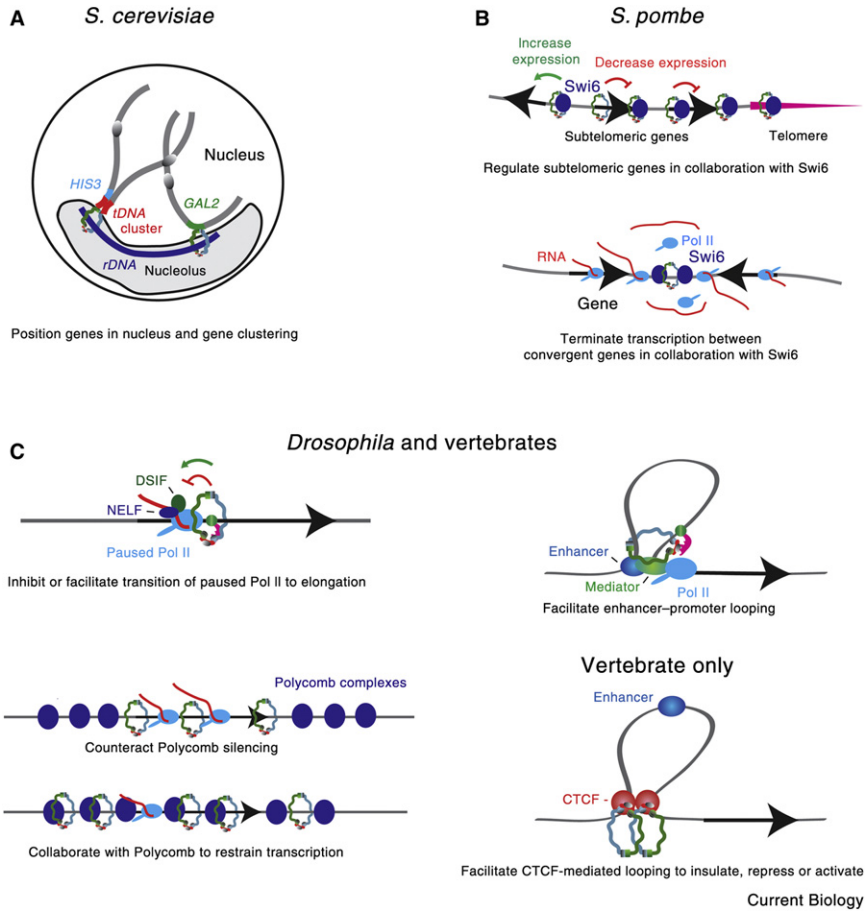

Figure 2. Roles of cohesin in gene expression.

(A) In *S. cerevisiae*, cohesin regulates genes by controlling their positioning within the nucleus, including proximity to the nucleolus and tDNA clusters. (B) In *S. pombe*, cohesin interacts with the Swi6 heterochromatin protein and together they regulate subtelomeric genes and increase transcriptional termination between convergent genes. (C) In *Drosophila*, and likely vertebrates, cohesin and adherin selectively bind genes with promoter-proximal paused RNA polymerase (Pol II) that also bind the DSIF and NELF pausing complexes. In a context- and gene-specific manner, cohesin and adherin modulate the transition of Pol II to elongation by unknown mechanisms. Adherin and cohesin facilitate enhancer–promoter looping, and can counteract silencing by Polycomb Group proteins, and more rarely, cooperate with Polycomb proteins to restrain, but not silence genes. In vertebrates, cohesin has likely, substituted for other CTCF cofactors seen in *Drosophila*, and directly interacts with CTCF to facilitate looping between CTCF binding sites, which can contribute to transcription repression, activation and insulation.

Cohesin, Heterochromatin and Gene Silencing

Cohesin binds heterochromatin in centromeric and telomeric regions, and functionally interacts with proteins that bind these regions in organisms from yeast to man. In *S. cerevisiae*, cohesin also binds the silent mating type loci, and cohesin mutations allow SIR silencing proteins to spread beyond the normal boundary that flanks the *HMR* silent mating type locus [51]. The *HMR* boundary forms at a tRNA gene promoter, which is required together with a nonenzymatic portion of the SIR2 histone deacetylase for sister cohesion at this site, in part because the TFIIIC transcription factor recruits kollerin [52–56]. Even with topologically bound cohesin at *HMR*, SIR2, but not silencing, is required for cohesion, suggesting that SIR2 directly participates in cohesion [53,56].

Despite this intimate relationship between cohesin and SIR2, cohesin does not contribute to silencing. In contrast to *sir2* mutations, inactivation of cohesin in G1, and adherin mutations do not derepress silenced genes [57,58]. An adherin missense mutation, however, affects expression of the *GAL2* gene that is positioned closely to the nucleolar rDNA repeats, some of which are silenced, and expression of a *HIS3* reporter gene positioned near the tRNA gene clusters that form adjacent to the nucleolus (Figure 2) [57]. In this adherin mutant, *GAL2* is easier to induce, and *HIS3*, which is repressed by tDNA clustering, increases in expression. Altered nucleolar morphology and reduced tDNA clustering accompany these expression changes. Also, many of the genes dysregulated by cohesin inactivation in G1 are situated adjacent to each other [58]. These findings argue that many of the effects of cohesin on gene expression in *S. cerevisiae* stem from repositioning of genes to new locations in the nucleus.

In *S. pombe*, cohesin interacts with the Swi6 heterochromatin protein 1 (HP1) homolog, which is required for cohesin to bind to the pericentric domains [59,60]. Adherin and cohesin mutations alter expression of few genes in fission yeast arrested in G1, but remarkably, these are clustered in subtelomeric domains near the Swi6-bound telomeres (Figure 2) [61]. Cohesin is required for Swi6 binding to the telomere and subtelomeric region, and cohesin and *swi6* mutations have similar effects on gene expression. The genes closest to the telomere increase in expression, while the more distal genes decrease. It remains to be determined if these effects correlate with changes in telomeric clustering or positioning, akin to the effects of cohesin on gene expression in *S. cerevisiae*.

Cohesin and Swi6 also affect transcription during G2 in *S. pombe*. Double-stranded RNA created by transcription through the 3′ ends of convergent genes leads to formation of Swi6 and cohesin binding regions between convergent genes through RNAi-dependent mechanisms, and subsequent transcriptional termination in these regions (Figure 2) [18]. Cohesin mutations reduce termination, indicating that cohesin contributes to termination in an unknown manner.

There is less evidence that cohesin regulates genes through heterochromatin-related mechanisms in higher eukaryotes. In human facioscapulohumeral dystrophy (FSHD), a reduction in the number of repeats of a 3.3 kb sequence (D4Z4) in a subtelomeric region of chromosome 4 leads to reduced histone H3K9 methylation, a heterochromatic modification, and reduced HP1γ and cohesin binding to the repeats, but whether or not this cohesin loss alters gene expression is unknown [62]. Mammalian heterochromatin proteins do not appear to be required for cohesin

binding to pericentric regions, although it has been reported that mammalian NIPBL interacts with HP1 proteins, and that HP1γ depletion reduces recruitment of NIPBL to DNA breaks [63–65]. Adherin and cohesin do not co-localize with HP1 at any of the prominent HP1-binding regions along chromosome arms in *Drosophila*, indicating that HP1 does not recruit them [20,21]. Thus, although cohesin concentrates in heterochromatic regions in higher organisms as in fungi, it may have less direct functional interactions with heterochromatin proteins.

Cohesin does show functional interactions with the Polycomb group (PcG) epigenetic silencing proteins in *Drosophila*. *Drosophila* cohesin paradoxically interacts with the PRC1 PcG complex in nuclear extracts, but is largely excluded from PcG-silenced regions on chromosomes, as detected by the histone H3 lysine 27 trimethyl (H3K27me3) mark made by the PRC2 complex (Figure 2) [21,66]. This exclusion is consistent with the isolation of *verthandi* Rad21 cohesin subunit mutations in a screen for genes that counteract PcG silencing [67,68]. Importantly, however, there are rare instances in which extended PcG-targeted domains are also coated with cohesin (Figure 2) [33]. These regions invariably contain genes encoding transcription factors that regulate development, such as *engrailed*. They are not silenced, but increase dramatically in expression upon depletion of adherin, cohesin, or PcG proteins. Thus, in contrast to regions targeted only by PcG proteins, these domains require both cohesin and PcG complexes to maintain a lower, restrained level of gene expression. Genome-wide analysis reveals that these genes are among the most sensitive to cohesin dosage.

The rare cohesin–PcG co-targeted genes in *Drosophila* are similar to bivalent genes in mammalian embryonic stem (ES) cells, which have both the PRC2 H3K27me3 modification and the H3K4me3 mark associated with active promoters. Some 70% of the genes in mouse ES cells that increase the most in expression with cohesin depletion are bivalent [24,69]. Like the cohesin–PcG target genes in *Drosophila*, bivalent genes largely encode transcription factors and other proteins that control development. Because cohesin is required to maintain the multipotent state of ES cells, it remains to be determined how many of the bivalent genes whose expression is induced upon cohesin depletion reflect direct repression of these genes by cohesin in combination with PcG proteins, as opposed to increases caused by differentiation. Nonetheless, these findings argue that cohesin and PcG silencing proteins play interlinked roles in development and differentiation, opposing each other in many cases, and cooperating in others.

Cohesin Selectively Binds and Regulates Genes with Paused RNA Polymerase

Clues to how cohesin and PcG proteins regulate genes in *Drosophila* arise from the findings that both preferentially bind genes in which RNA polymerase pauses after transcribing several nucleotides (Figure 2) [70,71]. The DSIF (DRB sensitivity inducing factor) and NELF (negative elongation factor) complexes associate with paused RNA polymerase II (Pol II), and release of Pol II from the paused state controls gene expression during development [72]. It remains to be seen if cohesin also selectively binds genes with paused RNA polymerase in vertebrates; however, cohesin does bind a pausing site in Kaposi's sarcoma-associated herpesvirus (KSHV) [73].

Some of the genes with paused polymerase decrease in expression upon cohesin depletion, though others increase, indicating that the effect of cohesin is context-dependent. Some of the genes that increase the most in expression upon cohesin depletion are the rare cohesin–PcG co-targeted genes, suggesting that cooperating repressor proteins may be one factor that determines whether cohesin activates or represses transcription.

Cohesin is not required for pausing, although it binds close to paused polymerase [71]. Its effects on expression also differ from those of the DSIF and NELF pausing factors. At strongly repressed genes, including some co-targeted by PcG proteins, cohesin and pausing factor co-depletion experiments indicate that cohesin interferes with the transition of paused polymerase to elongation at a step different from those controlled by the pausing factors. Cohesin is unlikely to simply obstruct polymerase movement, considering that it increases expression of many genes that it binds and that cohesin depletion does not increase the rate of transcriptional elongation along the *ecdysone receptor* (*EcR*) gene, which binds cohesin over much of the 80 kb transcription unit [71].

Pausing release requires phosphorylation of NELF, DSIF and the Pol II carboxy-terminal domain by the Ckd9 subunit of P-TEFb, which is recruited by transcriptional activators [74,75]. Considering the current evidence, therefore, some likely scenarios are that cohesin represses by interfering with modification of the transcriptional machinery, pausing factor release, or binding of elongation factors. Indeed, although they are transcribed, a notable feature of cohesin-binding genes is that they lack the H3K36me3 mark made by the Set2 protein that binds elongating polymerase [71,76]. In genes activated by cohesin, such as the *myc* gene in *Drosophila* and vertebrates [77], cohesin could facilitate transition to elongation by affecting the binding or activity of the same or different elongation factors in a context-dependent manner.

Cohesin Facilitates Enhancer–Promoter Looping and Transcriptional Activation

One mechanism by which cohesin can facilitate transition of paused polymerase to elongation is by increasing enhancer–promoter communication (Figure 2). *Drosophila* Nipped-B facilitates activation of the *cut* and *Ultrabithorax* homeobox genes by enhancers located some 80 and 50 kbp from their promoters [44]. Chromosome conformation capture (3C) experiments show that adherin and cohesin also facilitate enhancer–promoter contact and activation of pluripotency genes in mouse ES cells, β-globin genes in mouse/human erythroleukemia cells and fetal mouse liver, and the *Tcra* T-cell receptor gene in mouse thymocytes [24,49,78]. Adherin and cohesin associate with the enhancers and promoters, and reducing their dosage decreases looping and gene expression. Importantly, cohesin binding and enhancer–promoter looping are specific to cells in which the genes are active. The mechanisms by which cohesin facilitates enhancer–promoter contacts are unknown, although one obvious idea is that cohesin holds them near to each other in the same way it holds sister chromatids together (Figure 2).

In mouse ES cells, cohesin interacts with the Mediator complex and co-localizes with it at enhancers and promoters (Figure 2) [24]. Mediator binds RNA polymerase and regulates many aspects of transcriptional activation and repression, including enhancer–promoter looping, activator function, Pol II phosphorylation and elongation [79,80].

Thus, the cohesin–Mediator interaction may be involved in cohesin's control of the transition of paused polymerase to elongation and enhancer–promoter looping. Indeed, it raises the possibility that cohesin can regulate a gene by multiple mechanisms at the same time, and even have simultaneous positive and negative effects. Coinciding opposing effects might explain why reducing adherin dosage has an effect opposite to reducing cohesin dosage on expression of the *cut* gene in the developing *Drosophila* wing margin, or why small reductions in cohesin dosage decrease expression of *Enhancer of split* genes, and larger reductions increase their expression [33,45].

Consistent with the idea that cohesin regulates transcriptional activation and enhancer–promoter looping is the burgeoning evidence that cohesin associates with diverse cell type-specific transcription factor binding sites. Upon stimulation of breast cancer cells by estrogen, cohesin co-localizes with many estrogen receptor binding sites, and cohesin depletion alters the cellular response to estrogen [81]. Cohesin also associates with liver-specific transcription factor binding sites in human hepatocellular carcinoma cells [81]. Not only does cohesin maintain mouse ES cell pluripotency by facilitating enhancer–promoter looping and activation of pluripotency genes such as *Nanog* and *Oct4*, it also associates with many Nanog, Oct4 and Sox2 protein binding sites [24,26]. Nanog interacts with cohesin and the Wapl releasin subunit, suggesting that direct recruitment by activators is one mechanism by which cohesin selectively binds to specific genes. Upon differentiation of mouse ES cells, cohesin shifts to CTCF sites and sites bound by differentiation-specific transcription factors [26].

Cohesin and CTCF

CTCF is a zinc finger DNA-binding protein that functions in transcriptional repression, activation, and as an insulator that interferes with enhancer–promoter interactions [82]. Cohesin and CTCF interact, co-localize, and function together to regulate transcription [24–27]. Loops form between many sites that bind CTCF, and cohesin depletion reduces looping, with correlating effects on gene expression (Figure 2) [83–89]. For instance, cohesin depletion diminishes insulation by the *H19-Igf2*, chicken β-globin locus, and *MHC II C1* CTCF insulators [27,30,90].

A recent study using mouse embryonic fibroblasts, which strongly express *H19* and *Igf2*, found that cohesin or CTCF depletion did not reduce insulation at the imprinted maternal locus, but increased *Igf2* expression from the paternal locus, suggesting that CTCF and cohesin act as repressors [91]. CTCF and cohesin depletion also did not reduce imprinting at other loci, but increased transcript levels in some cases. Because increased expression of both the maternal and paternal *Igf2* alleles occurred in the absence of the CTCF binding sites on the maternal allele, it remains unclear if the repressive effects of CTCF and cohesin are direct.

The unexpected non-allelic effects of CTCF and cohesin on *Igf2* expression might reflect a role for CTCF–cohesin binding sites in the region surrounding the *H19-Igf2* locus in overall chromatin organization, similar to what is proposed for the human β-globin locus, where 3C analysis showed reduced cell type-specific looping between several CTCF binding sites, and reduced expression of the fetal γ-globin gene upon cohesin and CTCF depletion [85].

Cohesin's role in CTCF function is specific to vertebrates. *Drosophila* CTCF recognizes the same DNA sequence and insulates, but does not require, cohesin, relying instead on other factors such as the CP190 zinc finger/BTB co-insulator protein [92–94]. The CTCF accessory factors in *Drosophila* presumably do not bind DNA topologically, and thus CTCF function in vertebrate cells might not require topologically bound cohesin.

Evolution of Cohesin's Roles in Gene Expression and Development

Cohesin has acquired additional roles in gene regulation as organism complexity increased (Figure 2). It is not surprising, therefore, that given the developmentally important genes it controls, and the number of ways it exerts these influences, that even modest disruption of cohesin function alters many aspects of human development. What remains unclear is how much each of these activities are mechanistically related to each other, and reflect cohesin's ability to hold two DNA molecules together, or if new specific functional interactions with basal transcriptional machinery, activators and insulator proteins have arisen during evolution.

DNA Repair is an Ancient Cohesin Function

Two different strategies are used to repair a DNA double strand break (DSB) depending on cell type and phase of the cell cycle. Non-homologous end joining (NHEJ), used primarily in G1 phase, results in re-ligation of the broken DNA, and frequently leads to loss of genetic information, while homologous recombination (HR) depends on a homologous DNA template, and thus is preferentially performed during the S and G2 phases using a sister chromatid template [95]. Because sister chromatids are identical, HR leaves genetic information intact [96]. The finding that DNA repair efficiency increases when yeast cells go from G1 to G2 argues that completion of replication, i.e. formation of sister chromatids, is important for repair [97]. In addition, HR requires close proximity between the broken DNA and the repair template; therefore, the importance of sister chromatid cohesion for repair was predicted [98].

The DSB repair function of cohesin is ancient, inherited from its bacterial SMC protein ancestors [99]. An early indication of cohesin's role in DNA repair in eukaryotes, predating the discovery of its role in sister chromatid cohesion, was that a mutated Rad21 cohesin subunit rendered *S. pombe* cells sensitive to γ-irradiation and defective in DSB repair [100]. In addition, a mammalian SMC1/3-containing complex was demonstrated in biochemical experiments to facilitate certain types of DNA repair [101]. Studies in Rad21-depleted chicken DT40 cells, cell lines from breast cancer patients with impaired Rad21 function, and depletion of Rad21 in HeLa cells confirmed that cohesin is also important for repair in higher eukaryotes [102–104].

Cohesin and Checkpoint Activation

Cell cycle checkpoint activation is the initial response to DNA damage, delaying cell cycle progression until genome integrity is restored [105]. Early evidence that cohesin is important for checkpoint activation came from human cell and mouse studies, when it was discovered that ATM (ataxia-telangiectasia mutated)- and NBS1 (Nijmegen Breakage Syndrome protein 1)-dependent phosphorylation of Smc1 and Smc3 is important for induction of the intra S phase checkpoint in response to irradiation (Figure 3) [106–109]. It was later found that cohesin is also involved in the damage-induced checkpoint in post-replicative human cells. The effector

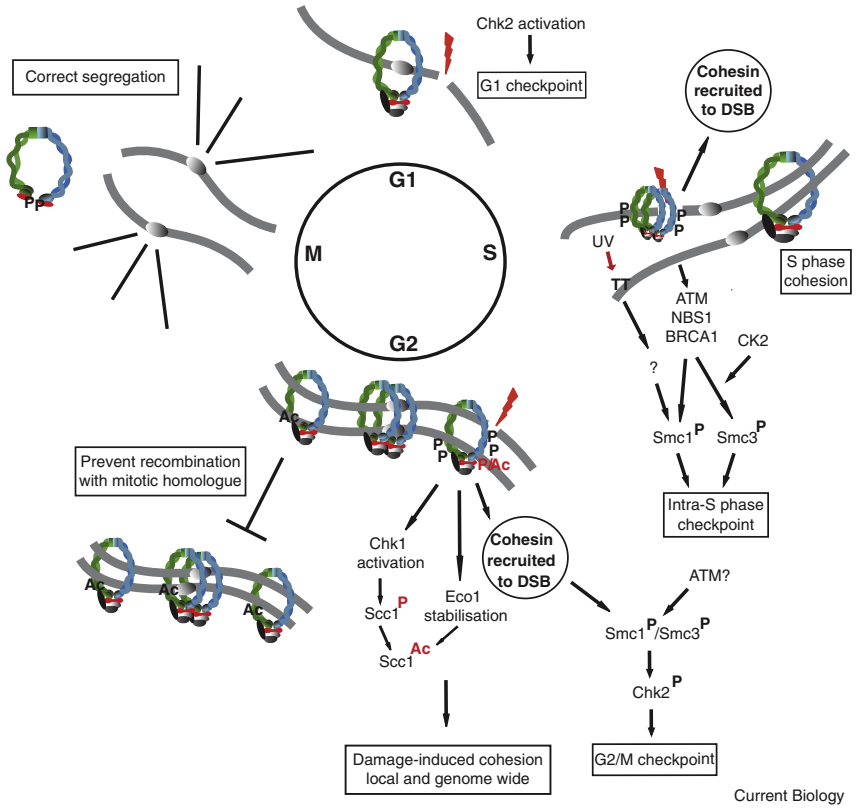

Figure 3. Functions for cohesin in DNA damage responses, DNA repair and genome integrity.

The different DNA damage response and repair actions that cohesin has been shown to be involved in are indicated in relation to the cell cycle. For further details see the main text. Phosphorylation, P; Acetylation, Ac.

function of Smc1/3 phosphorylation as it relates to the checkpoint is still unclear; however, depletion of Smc3 or Scc1 allows cells to proceed through the cell cycle after DSB induction, leading to a majority of cells with broken chromosomes [110].

One likely reason for the failure to activate the checkpoint properly in cohesion-deficient cells is inefficient activation of the Chk2 kinase. That Chk2 is not phosphorylated properly in G1 in the absence of Rad21, when there is no cohesion, suggests that cohesin, and not sister chromatid cohesion *per se*, is required for checkpoint activation. Supporting this idea, inactivation of Sororin, a metazoan-specific cohesion factor, prevents establishment and maintenance of cohesion, but not activation of the checkpoint [110]. In *Caenorhabditis elegans scc-2* adherin mutants, binding of cohesin to chromosomes and DNA repair are both diminished during meiosis [111]. Despite the lack of DSB repair, the DNA damage checkpoint is still not activated, arguing that activation requires chromosome-bound cohesin. So far, cohesin has not been reported to be directly involved in checkpoint signaling or maintenance in yeast.

Sister Chromatid Cohesion and Postreplicative Double-Strand Break Repair

Cohesin is not known to be required for checkpoint activation in response to a DNA break in yeast, but is required for repair. Studies by Nasmyth and colleagues in *S. cerevisiae* showed that DSB repair in G2 is impaired if DNA replication occurs in the absence of functional cohesin, and that reintroduction of cohesin during G2 cannot rescue this deficiency [98]. It was further concluded that sister chromatid cohesion, and not just cohesin binding, was important for repair because inactivation of Eco1, which blocks cohesion, but not cohesin binding, also causes repair deficits. Depletion of Sororin diminishes both cohesion and repair but not cohesin DNA binding, indicating that cohesion is also required for postreplicative DSB repair in mammalian cells [104].

Recruitment of Cohesin to DNA Breaks

The first indication that cohesin is recruited to damaged DNA came from a study where human cells were exposed to high doses of laser irradiation [112]. However, nuclear structure was severely disrupted and actual break localization may not have been demonstrated. Milder methods for inducing damage detected phosphorylation of SMC1 but not increased SMC1 levels at break sites [113]. Regardless, recruitment of cohesin to single site-specific DNA breaks has been confirmed in yeast and human cells (Figure 3) [114–116].

In *S. cerevisiae*, γ-radiation-induced breaks are not repaired if cohesin loading, and thereby localization to breaks, is inactivated in G2 after establishment of S phase cohesion [115,116]. Thus, despite proper S phase cohesion, cohesin has to bind at breaks for repair to occur. It was further demonstrated, taking advantage of temperature-sensitive S phase cohesin and wild-type cohesin expressed in G2, that DNA damage induces cohesion, termed damage-induced (DI) cohesion (Figure 3) [115,117].

Regulation of Damage-Induced Cohesion

In yeast, cohesin loading at a DSB, and establishment of cohesion on both damaged and undamaged chromosomes require both the DNA damage response pathway and several factors that regulate chromatid cohesion [115,116,118,119]. Thus, DI cohesion is not established in the absence of functional Eco1, which is also essential for cohesion establishment during S phase [118,119]. Cohesion establishment during S phase is coupled to DNA replication. Because DSB repair via HR triggers DNA synthesis, and Eco1 is required for DI cohesion, it was predicted that DI cohesion would also require DNA synthesis. However, deletion of Rad52, required for strand invasion during HR and thus DNA synthesis, does not reduce DI cohesion. Even more striking, DI cohesion is activated throughout the genome in response to a single DSB, demonstrating that cohesion can be established independently of DNA synthesis during G2 [118,119].

What signal is transmitted by a DSB to allow *de novo* cohesion establishment? Koshland and co-workers found that

the checkpoint kinase Chk1 is required for DI cohesion, and Chk1 was believed to phosphorylate a conserved serine residue (S83) of Scc1. Although phosphorylation of this residue could not be detected *in vivo*, a mutant that cannot be phosphorylated at S83 was unable to establish DI cohesion. S83 phosphorylation was suggested to augment acetylation of K84 and K210 residues in Scc1 by Eco1 [120]. During S phase, Eco1 acetylation of the Smc3 cohesin subunit counteracts the anti-establishment activity of Wpl1 [121–126]. Thus, Chk1 phosphorylation of Scc1 in response to DNA damage was suggested to help counteract Wpl1 (Rad61) to specifically establish postreplication cohesion. In addition, DNA damage during G2/M stabilizes Eco1 by preventing its phosphorylation by Clb2–Cdk1 and subsequent ubiquitin-mediated degradation, which may further increase modification of cohesin to counteract Wpl1 (Figure 3) [127].

Recent reports suggest that DI cohesion also occurs in multicellular organisms. A single DSB increases the proximity of sister chromatids in the region close to a DNA break in chicken DT40 cells [128]. Furthermore, alignment of sister chromatids is transiently enhanced in response to X-irradiation or mitomycin C in *Arabidopsis thaliana* [129]. This process may also be regulated by the same checkpoint as in yeast, given that Chk1 is constitutively phosphorylated in human cells with mutant Esco2 [130].

Is Local and Genome-Wide DI Cohesion Critical for DSB Repair?

A central question is to what extent DI cohesion contributes to DNA repair. Repair is abolished if Eco1 is inactivated, which prevents DI cohesion, but does not affect recruitment of cohesin to DNA breaks. This argues that DI cohesion is required for repair [118,119]. Newer data, however, indicate that this may be an oversimplification. Inactivation of other factors required for full formation of DI cohesion genome-wide, such as Tel1, Mec1, Chk1, and H2A phosphorylation, leaves DSB repair mostly unperturbed [131]. Because inactivation of adherin and Eco1 abrogates both DI cohesion and DSB repair, these proteins have other functions in repair besides establishing DI cohesion. These roles remain to be determined, but adherin is likely required for topological binding of cohesin at a DNA break, and it can be speculated that Eco1 modification of cohesin counteracts the propensity of releasin to remove this topologically-bound cohesin.

Removal of cohesin by separase during interphase appears to be required to complete DNA break repair in *S. pombe* [132]. Possibly, this transient or local removal of cohesion at a break site is why cohesion is reinforced genome-wide. This might also be the case in higher eukaryotes, as a recent study in human cells revealed that cohesin binding is reinforced genome-wide after irradiation [133].

Genome-wide DI cohesion may also prevent precocious sister chromatid separation during a G2/M arrest caused by checkpoint activation. This question has been addressed with different outcomes in budding yeast. The absence of functional Eco1 during G2, using a temperature-sensitive allele of *Eco1* (*eco1-1*) that gives a high background of precocious sister separation, did not increase chromosome mis-segregation, nor did a missense mutation, $eco1^{(W216G)}$, which mimics a human *ESCO2* Roberts syndrome mutation [118,134,135]. In contrast, a three-fold increase in loss of unbroken chromosomes was seen in the $eco1^{ack-}$ mutant strain after break induction, although the total frequency was very small [119].

As discussed above, cohesin also regulates gene expression, and thus one unexplored possibility is that DI cohesion may also be important for the characteristic transcriptional response to DNA damage [136,137].

Additional Functions for Cohesin in DNA Repair

Cohesin's role in DNA repair has mainly been attributed to its ability to hold sister chromatids together. This is beneficial for HR, where the preferred template for repair, the sister chromatid with identical sequence, is held in close contact with the broken DNA molecule. Indeed, a four-fold reduction of the Scc1 or Smc3 cohesin components decreases survival in response to irradiation and increases recombination between homologues, which augments the risk for loss of heterozygosity [138]. In addition to promoting repair from the sister chromatid, it has also been suggested that cohesin can regulate the choice between the HR and the NHEJ pathways for DSB repair [139]. Cohesin is also required for DSB repair during meiosis [140]. Here it is critical that the programmed DSBs created for initiation of meiotic recombination between homologous chromosomes are repaired via the homologous chromosome and not the sister chromatid [141,142]. In line with this, cohesion is relaxed in the immediate vicinity of meiotic DSBs, indicating that it is regulated differently in meiosis than in somatic cells [143].

Do DNA Repair Deficits Contribute to the Molecular Etiology of the Cohesinopathies?

Human developmental disorders, such as CdLS, caused by dominant mutations in adherin and cohesin subunits, and Roberts-SC phocomelia syndrome (RBS/SC), caused by loss-of-function mutations in both copies of the Esco2 ortholog of Eco1, are known collectively as the cohesinopathies [144]. As mentioned, many of the diverse developmental deficits in CdLS stem largely from gene dysregulation, but the molecular etiology of RBS/SC is poorly understood [145]. However, cells from both CdLS and RBS/SC individuals display increased sensitivity to DNA damage-inducing agents [37,40,130,146,147]. Deficiencies in DNA repair would be predicted to cause increases in the frequency of cancer, or even immune deficiencies. Currently, there are insufficient data to know if this is the case, but cancer is responsible for only 2% of deaths in CdLS [148]. An increase in cancer, if it occurs, may be small and masked by the more frequent causes of morbidity associated with the structural birth defects. There is even less information regarding RBS/SC, but an RBS/SC mutation recreated in budding yeast shows that Eco1 promotes reciprocal crossing over after treatment with the radiomimetic bleomycin during mitotic growth [135].

Do DNA Repair Deficits or Gene Expression Changes Arising from Cohesion Factor Mutations Contribute to Cancer Progression?

A hallmark of cancer is aneuploidy, and reinforcement of cohesion in response to DNA damage could be a way to prevent this. However, cohesion and chromosome segregation are the most robust of cohesin's functions, and require less than 20% of normal cohesin levels [32,33]. On the other hand, the NIPBL adherin is important for survival after ionizing radiation treatment, and Rad21 haploinsufficiency impedes DNA repair and enhances gastrointestinal radiosensitivity in mice [149–151]. In addition, *NIPBL* mutations

are found at high frequency in a panel of colon cancers, Rad21 alterations occur in breast, prostate cancer and leukemia, and Stag2 (SA) mutations are found in a variety of tumor types [103,152–155]. Thus, even in the absence of overt cohesion defects, impaired DNA repair could contribute to mutagenic processes. Recent studies in yeast indicate that aneuploidy can cause defects in DNA repair, and thus it can be speculated that aneuploidy enhances the repair deficits associated with cohesion factor mutations [156].

Finally, it is also possible that altered gene expression caused by modest reductions in cohesin function could contribute to cancer progression. It has also been proposed that overexpression of Rad21, associated with poor prognosis in breast cancer, may influence disease progression by changes in gene expression [157].

Concluding Remarks

As described above, like its role in chromosome segregation, cohesin's ancient role in DNA repair, inherited from its bacterial ancestors, has been retained throughout evolution. We cannot, however, be sure that some aspects have not changed until the detailed mechanisms are elucidated, and we also do not yet know to what extent cohesin's dosage-sensitive role in repair contributes to the etiology of cancer. It has also been revealed that cohesin has acquired more roles in gene regulation with increasing organismal complexity, to the point where even minor changes in cohesin activity can have drastic consequences for development. Elucidation of the mechanisms by which cohesin participates in DNA repair and gene regulation therefore remains of substantial relevance for human health.

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